Protocol for 51-Cr and 111-In Radiolabeling of Fresh Whole Blood Controls *en-tube*

FDA Workshop on Use of Radiolabeled Platelets for Assessment of In Vivo Viability of Platelet Products

Monday, May 3 2004
Lister Hill Auditorium-NIH Campus
Bethesda, MD

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Conflict of Interest Statement

- Pall Corp: BOD, MAB, Platelet Clinical Trials
- Cerus Corporation Clinical Trials: S-59; S-303
- Vitex Clinical Trials: Pen 110 Red Cells Phase II
- Baxter: Clinical Trials, Advisory Panels
- Mission Medical: Device/Radiolabeling Trials
- Haemonetics: Device/Radiolabeling Trials
- Terumo: MAB, Device/Radiolabeling Trials

- Total Corporate Equity $0.00 (= purity)

Study Purpose

- **Validate a dual plt radiolabeling protocol using 51-Cr and 111-In to radiolabel fresh autologous platelets, e*n-tube***
- **Based on protocols from Aubuchon Lab and Snyder Lab for 111-In and 51-Cr labeling**
- **Purpose:**
  - **Determine 51-Cr / 111-In e*n-tube* labeling efficiencies**
  - **Determine in vivo Recovery (%)**
  - **Determine in vivo Survival (hrs/days)**
  - **Validate that sampling to day 7 is adequate (vs D10)**
  - **Determine % of CONTROL value to be used as acceptable for TEST Recovery/Survival studies**
- **Analyze with COST Program**

Donor Processing

- **IRB approval; RSC approval**
- **Recruit nl volunteer donor; TTD/Preg tests**
- **Unknowns:**
  - **quantity of platelets needed**
  - **volume of blood necessary**
  - ***en-tube* labeling efficiency of 51-Cr**
  - **% 51-Cr elution when labeled *en-tube***
  - **equivalency of 111-In with 51-Cr e*n-tube* labeling**
  - **51-Cr recovery/survival characteristics at low L.E.**
  - **window settings; xtal size; counting time; sample days**

Whole Blood Processing

- Using a 19 g needle and 60 mL polypropylene syringe(s) containing 7 mL of ACD-A, collect 43 mL of venous blood (50 mL total)
- ACD-A adjusted to volume of blood collected
- 100-125 mL whole blood collected for both labels
- Transferred contents to 50 mL conical tubes and gently mixed.
- Whole blood left undisturbed at room temperature for 1 hour

Platelet Rich Plasma Preparation

- Soft spin conical tubes at 200 x g for 15 minutes in a swinging bucket centrifuge at 20°-24°C to prepare red cell-poor, platelet rich plasma (PRP)
- Remove PRP with 18g spinal needle (can spin x 2)
- Avoid aspirating red cells
- Add a volume of sterile ACD solution equal to 15% of the PRP volume and mix gently by inversion
- Platelets split, 60% for chromium, 40% for indium

PRP Preparation

- Centrifuge the ACD-PRP at 2000 x g for 15 minutes with brake off – for both labels
- Remove platelet poor plasma (PPP) as completely as possible and save
- Resuspend harvested platelet pellet with 3 mL ACD-A/saline solution in a conical polypropylene tube

111-In Platelet Labeling

- **Add 100 µCi of ^{111}Indium Oxine in 4 mL of ACD-A /saline to the washed platelet pellet**
- **Gently resuspend the platelet pellet**
- **Incubate at 20°-24° C for 25 minutes**
- **Mix gently at 10 minutes**

51-Cr Platelet Labeling

- **Add 200 µCi of ^{51}Sodium Chromate to PRP**
- **Gently resuspend the platelet pellet**
- **Incubate at 20°-24° C for 25 minutes**
- **Mix gently at 10 minutes**

Further Labeling

- After incubation, add 0.5 mL of autologous PPP and 3.5 mL ACD/saline to the platelet suspension
- Centrifuge platelet-ACD/saline suspension at 2000 x g at 20°–24° C for 10 minutes.
- Remove supernatant and save in a separate tube
- Determine the activity of supernatant in a dose calibrator

Labeling Efficiency

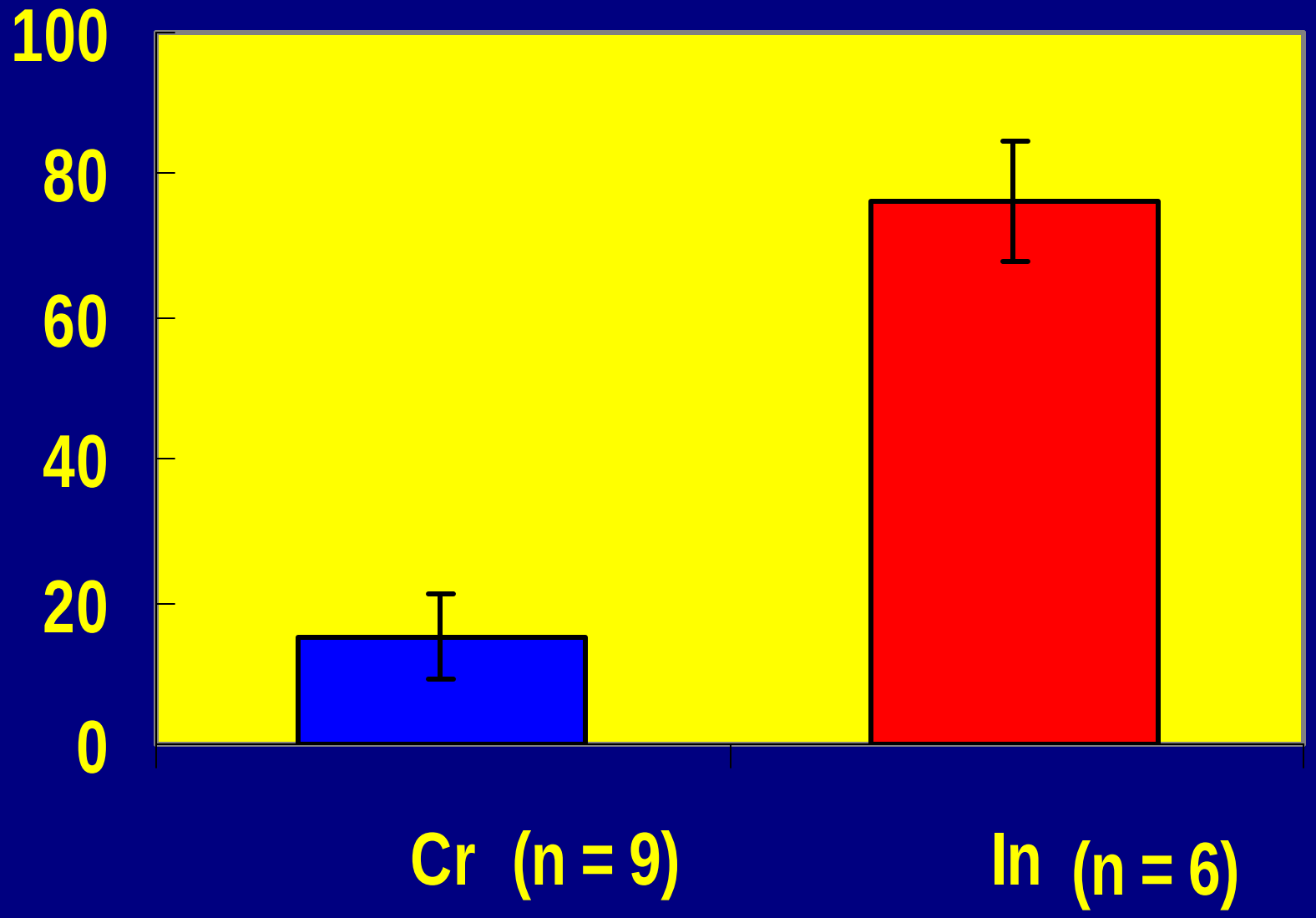
- Gently resuspend the platelet pellet in 6 mL of autologous PPP
- Determine the exact activity of the ^{111}Indium- or ^{51}Chromium-labeled platelets for injection using a dose calibrator to calculate labeling efficiency

- Labeling Efficiency =

$$\frac{\text{ActivityPlatelets}}{\text{ActivityPlatelets} + \text{ActivitySupernatant}} \times 100$$

- Aspirate a volume of labeled platelet concentrate containing up to 40 µCi in a 3-10 mL plastic syringe using an 18 g spinal needle.

Labeling Efficiency (%)



Preparation of Standards

- Prepare a Standard for determination of radioactivity in the infusate
- Prepare a 1:2500 dilution of 51-Cr and 111-In labeled platelets by adding exactly 0.1 mL into a 250 mL volumetric flask and q.s. with H_20 to 250 mL
- Transfer exactly 2 mL aliquots to each of the 3 counting vials for each isotope

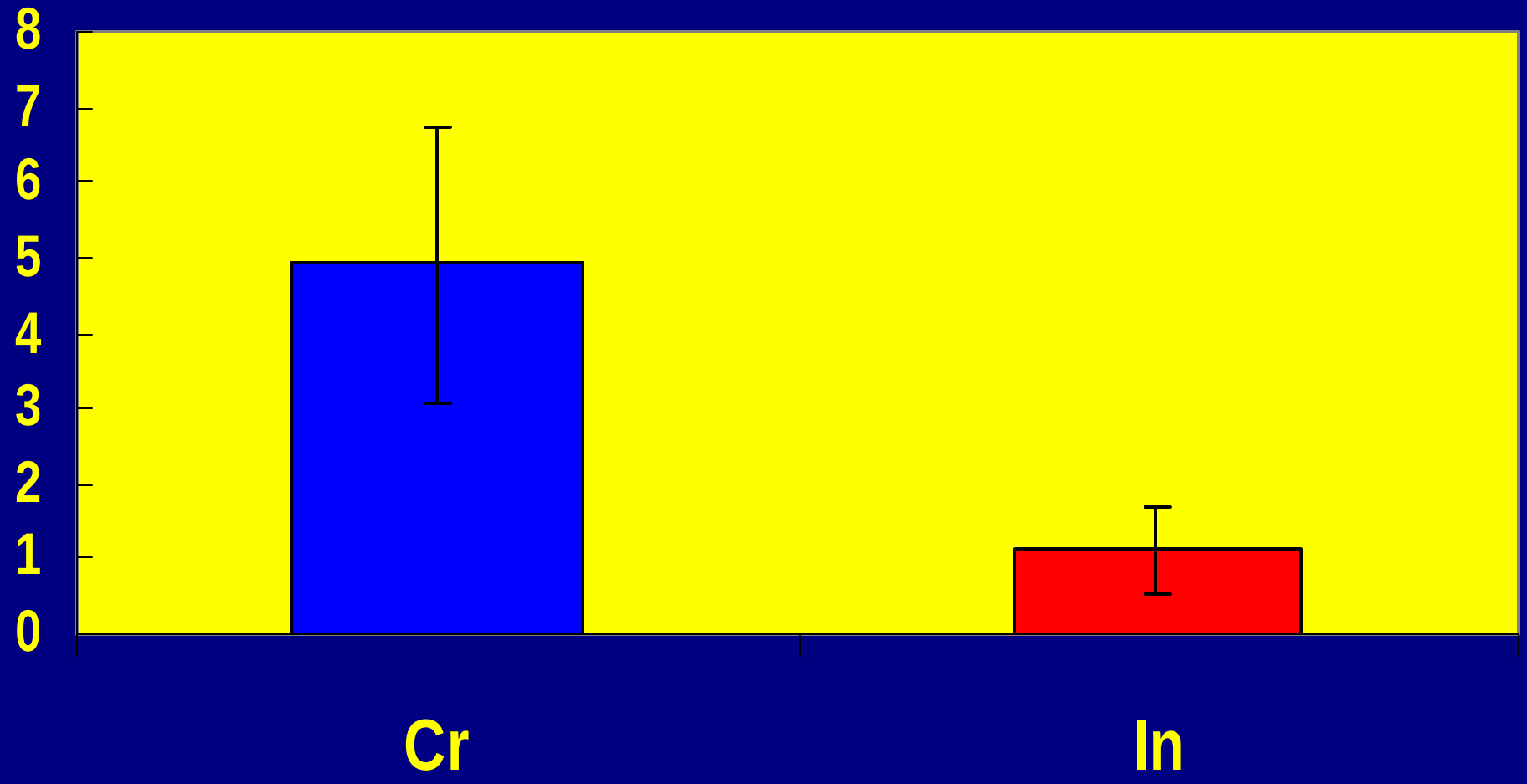
Preparation of Elution Samples

- **Incubate the remaining injectate in autologous plasma at 22°C for 2 hours; time from when injectate is prepared**
- **After 2 hour incubation, mix the platelet sample well**
- **Transfer approximately 1 mL of the platelet sample to a polypropylene microcentrifuge tube (min 200 μL)**
- **Centrifuge platelet sample aliquot at approximately 10,000g (maximum speed) for 2 minutes**

Preparation of Elution Samples

- Prepare 2 elution samples - for each, transfer 100 µL of the supernatant to a counting vial without disturbing the platelet pellet
- Add 1.9 mL water or saline to each supernatant aliquot; bring to a volume of 2 mL
- Prepare background tubes in duplicate by adding 2 mL water to 2 counting vials
- Count 2 background samples, 2 elution supernatant samples, and 3 platelet standard samples for each isotope in a gamma well counter

Elution (%)
(n = 6)



Elution Calculation

Formula for calculation of % isotope elution

% Elution =

$$\frac{\textbf{(Average CPM elution supernatant – Average Background)}}{\textbf{125* X (Average CPM Platelet Standard – Average Background)}} \textbf{ X 100}$$

* correction factor as per Cerus Corp

Sample Injection

- Perform venipuncture using a 19g butterfly infusion set and 3-way stopcock
- Collect 2 10mL purple top baseline tubes
- Ensure vein patency

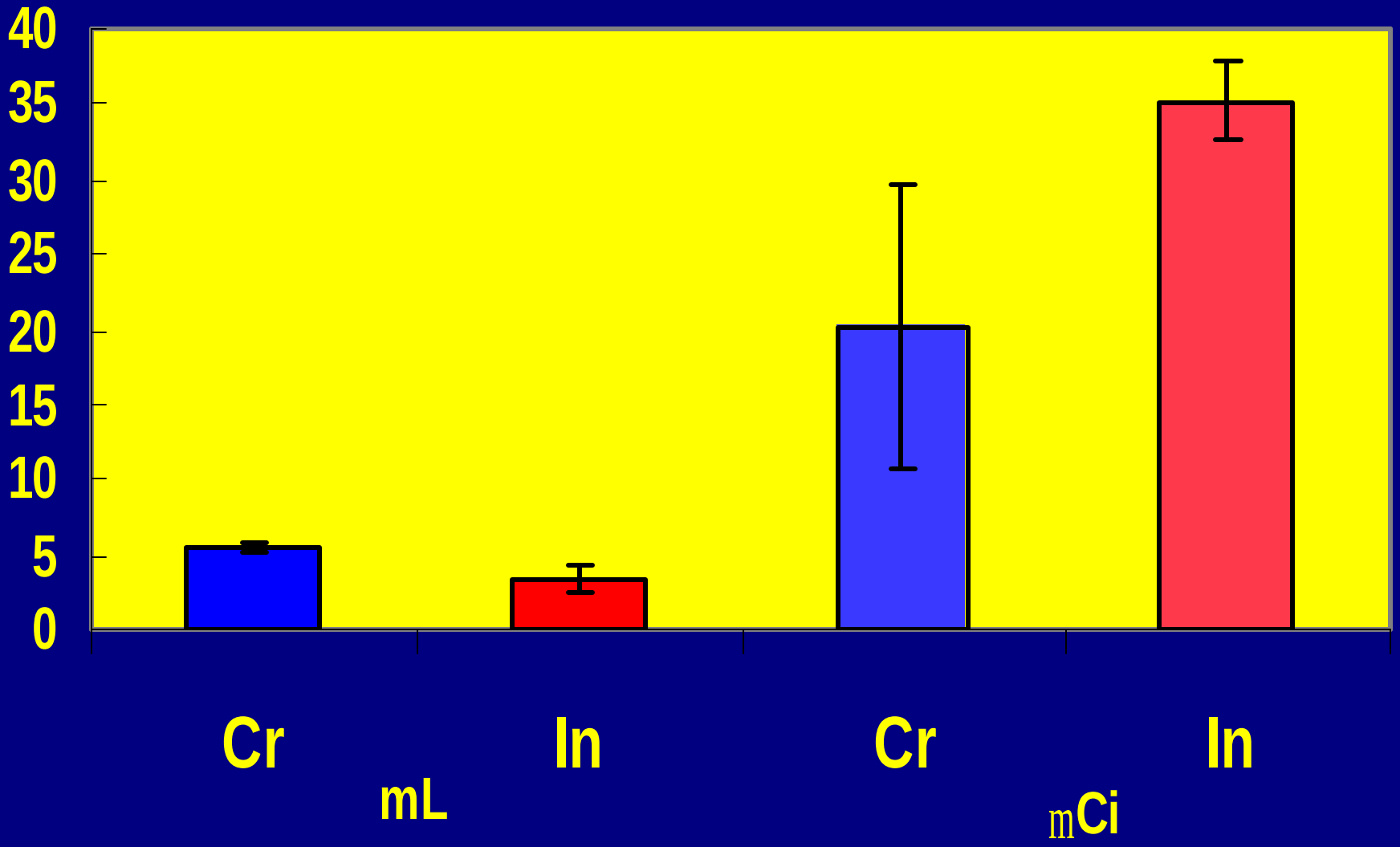
Sample Injection

- We infused indium first (adsorbs to surfaces)
- Flush tubing and empty syringe x 2
- Attach second (Cr) syringe and infuse
- Flush tubing and empty syringe x 2
- Check residual radioactivity of syringes

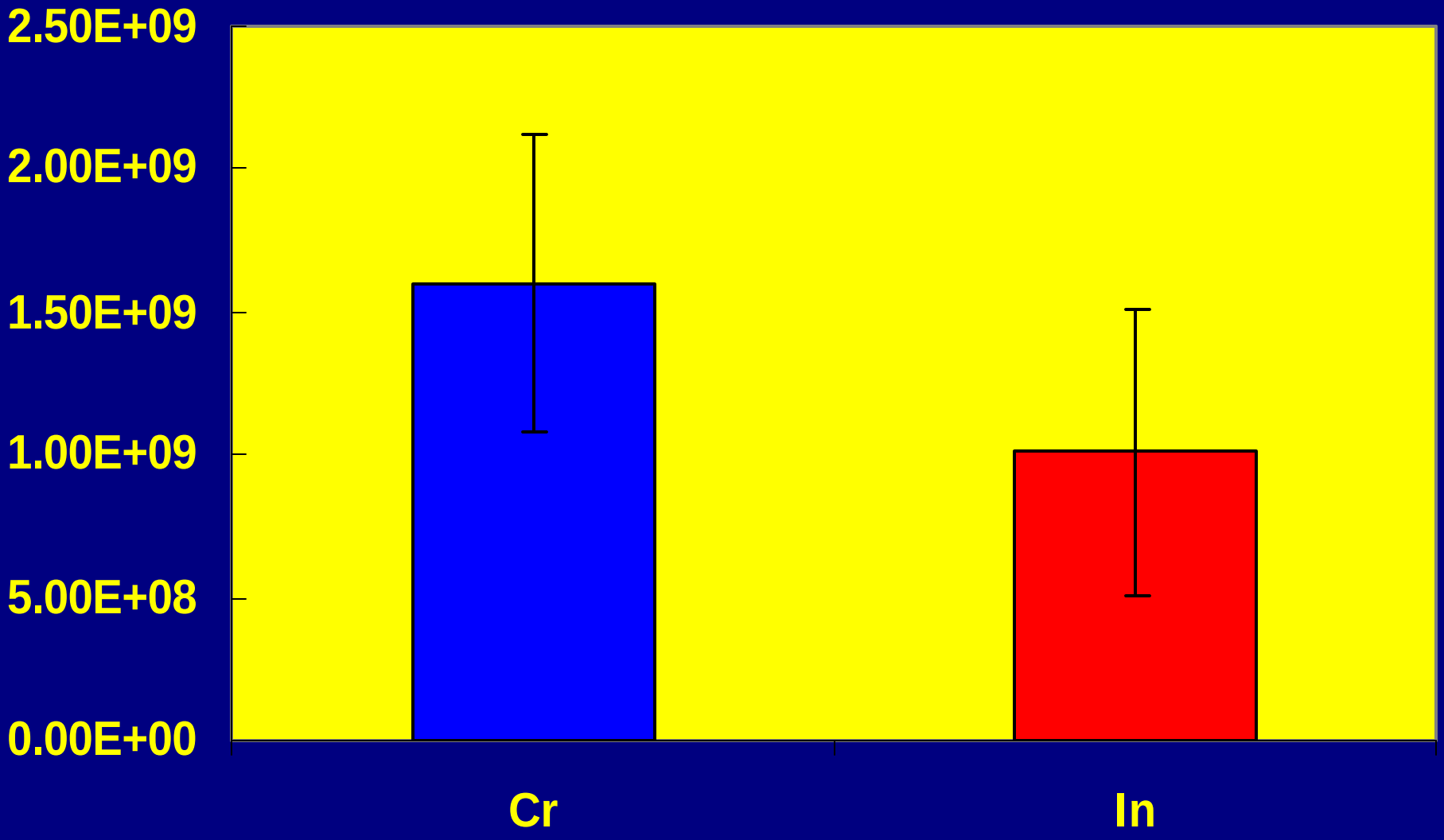
Amount Injected

(n = 6)

(40 µCi targeted)



Total Platelets Injected
(n = 6)



Sample Collection

Collect 2 10 mL purple top tubes at:

- 1, 2 and 3 hours post infusion
- 24 hr (+/- 2 hours)
- Daily on days 2-7 and 10 (not Sunday)

Sample Processing

- **Draw two 10 mL purple top samples**
- **Aliquot 2-2 mL whole blood samples into counting tubes (sample from both tubes)**
- **Hard spin residual blood in 10 mL tubes at 2000 x g for 15 minutes**
- **Aliquot 2-2 mL supernatant samples into counting tubes (sample from both tubes)**
- **Store at room temperature**

Counting

- Samples counted in duplicate on a Wallac/Perkin Elmer Model 1470 (2”x1” NaI xtal)
- Windows set to count In/Cr simultaneously (**5 min**)
- In window settings 165-215 KeV (171, 247, 419-Sp)
- Chromium window settings were 295-340 KeV
- Counter software adjusts for decay / background
- Includes only counts within selected range for cpm

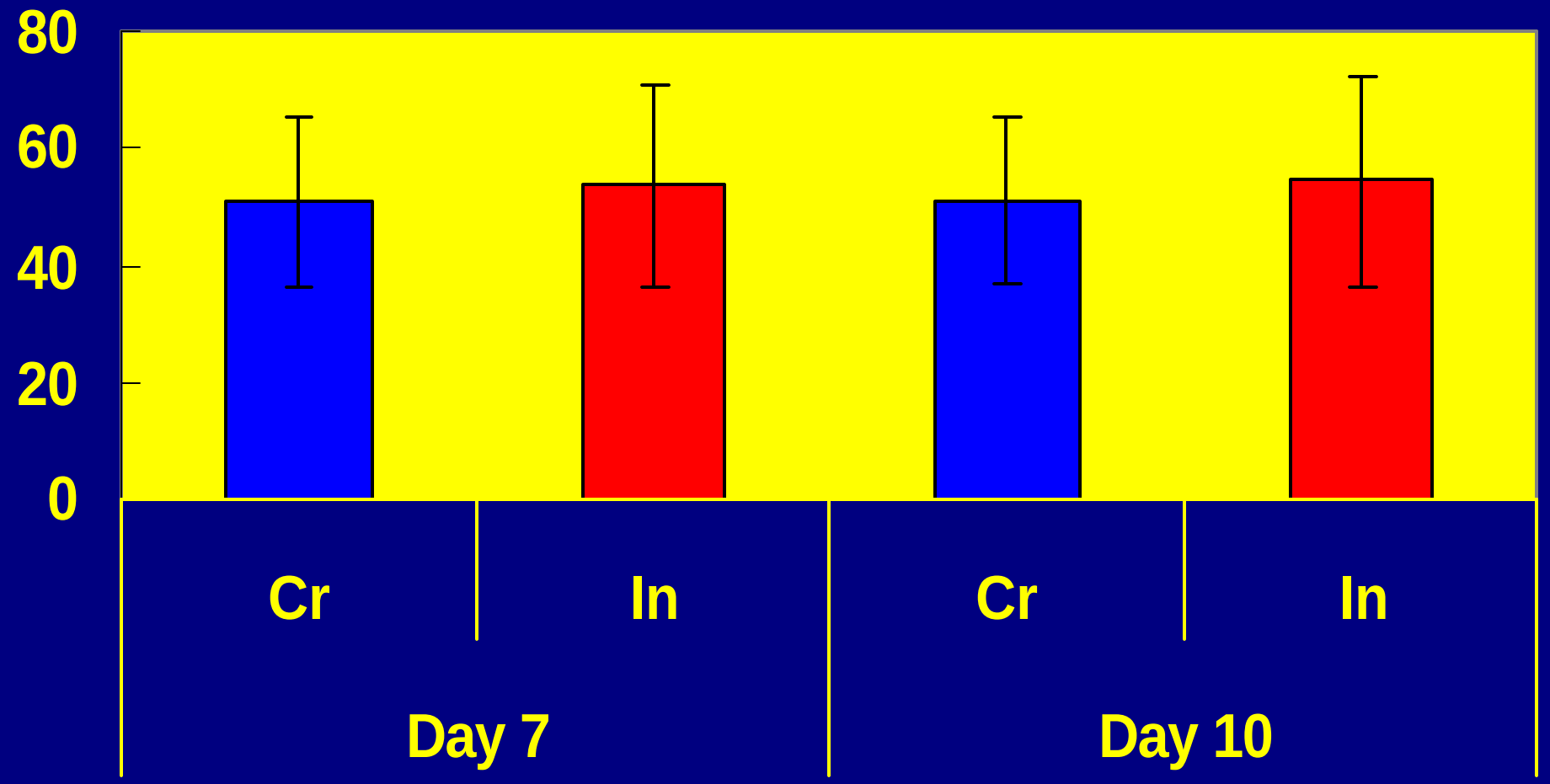
In Vitro Radiolabeling Data Analysis

vol coll (mL)	Plt ct/?L x10E3	Starting Cr (?Ci)	Starting In (?Ci)	Cr % label eff	In % label eff	Cr mL inj	Cr ?Ci inj	In mL inj	In ?Ci inj	Cr Elution	In Elution	Cr total plt inj	In total plt inj
50	333	200	ND	16									
50	339	200	ND	16									
50	218	196	ND	14									
100	398	196	120.7	24.6	71.8	5.4	31.9	5	36.8	3.2	2	2.15E09	1.99E09
100	246	193	115.6	15.5	71.4	6	21.9	3	32.9	4.8	1	1.48E09	7.38E08
125	415	200	117	22.5	71.2	5.4	30	2.8	33	3.8	0.6	2.46E09	1.28E09
125	253	197	117	11	85	5.4	15.4	3	36.2	3.4	0.5	1.37E09	7.59E08
125	205	200	113.6	10	88.7	5.6	12.8	2.2	33.2	7.6	1.2	1.15E09	4.51E08
125	199	200	116.8	6.0	69.2	5.2	8.9	4.2	39.2	6.7	1.4	1.03E09	8.36E08
MEAN	289.6	198.0	116.8	15.1	76.2	5.5	20.2	3.4	35.2	4.9	1.1	1.6E09	1.01E09
STD DEV	83.3	2.6	2.3	5.9	8.4	0.3	9.4	1.0	2.6	1.8	0.6	5.2E08	5.02E08

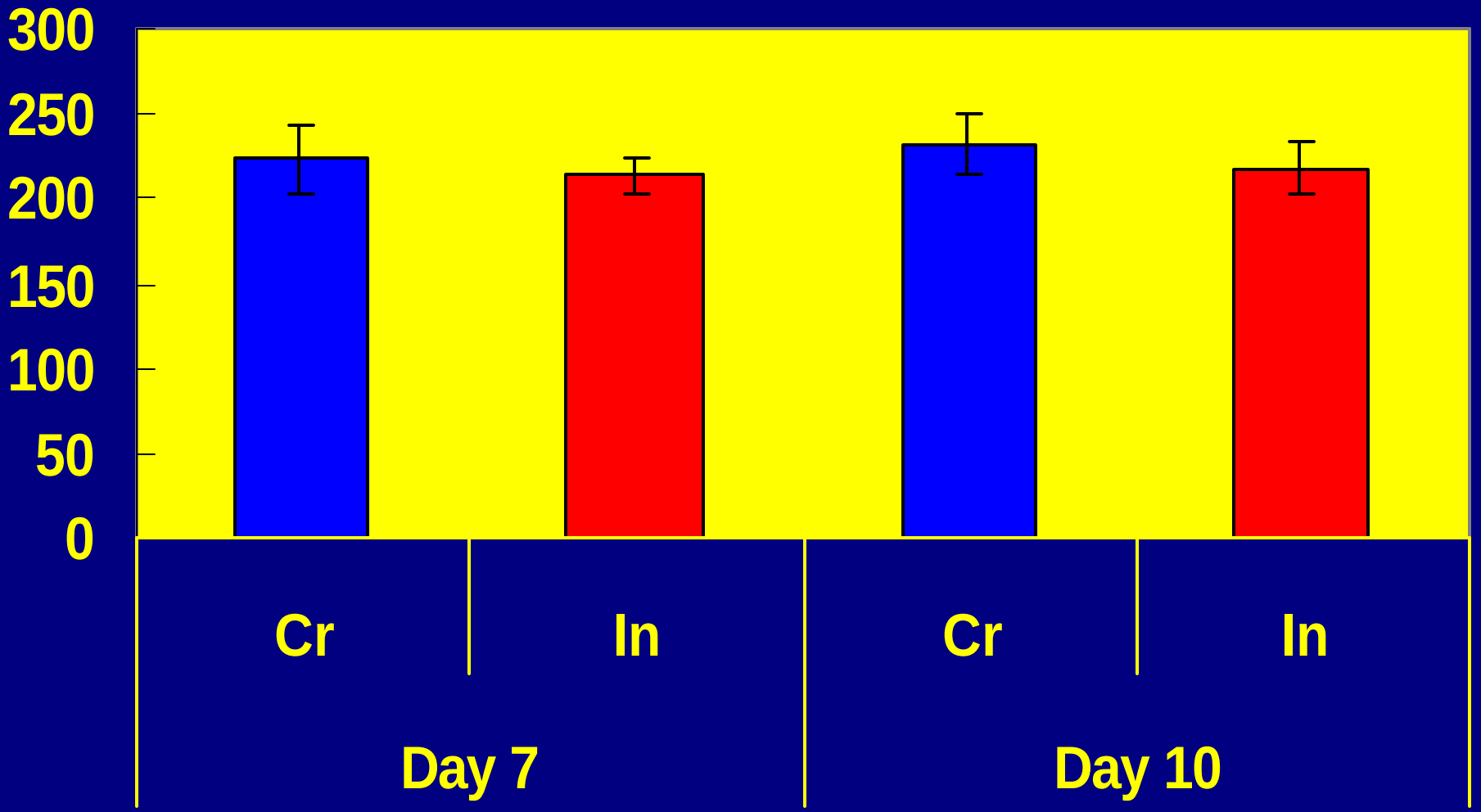
Snyder Lab Recovery and Survival Data (n=6)

Subject	Sex	day 7 recovery (%)		day 7 survival (hrs)		day 10 recovery (%)		day 10 survival (hrs)	
		Cr	In	Cr	In	Cr	In	Cr	In
D (DF)	F	51.01	63.09	227.6	221.6	53.53	63.55	245.3	217.9
E (DP)	F	68.9	69.88	250.2	219.5	65.6	69.1	257.7	224.1
F (GC)	M	60.7	68.19	225.3	224.8	62.23	69.93	230.2	223.8
G (DC)	F	26	27.25	230.1	213.8	26.17	26.75	228.6	223.6
H (GM)	F	52.79	55.55	214.1	210.5	53.7	59.6	221.8	229.3
I (ES)	F	45.6	37.58	192.4	195.2	46.01	37.38	206.8	187
MEAN		50.83	53.59	223.28	214.23	51.21	54.39	231.73	217.62
Std Dev		14.64	17.45	19.14	10.68	14.10	18.01	17.84	15.43

Recovery (%)
(n = 6)



Survival (hrs)
(n = 6)



Snyder Lab Recovery and Survival Data (n=6)

Subject	Sex	day 7 recovery (%)		day 7 survival (hrs)		day 10 recovery (%)		day 10 survival (hrs)	
		Cr	In	Cr	In	Cr	In	Cr	In
D (DF)	F	51.01	63.09	227.6	221.6	53.53	63.55	245.3	217.9
E (DP)	F	68.9	69.88	250.2	219.5	65.6	69.1	257.7	224.1
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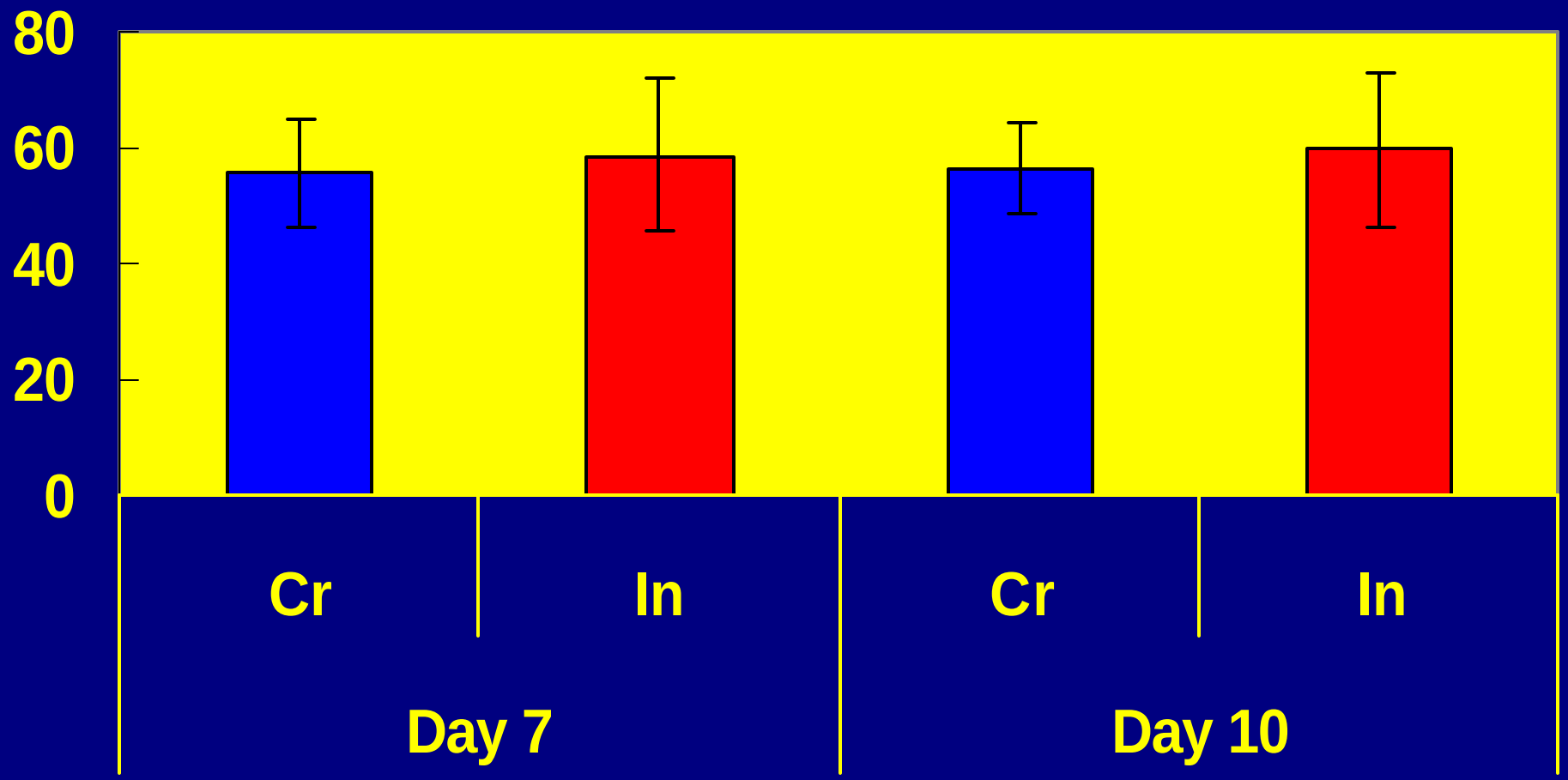
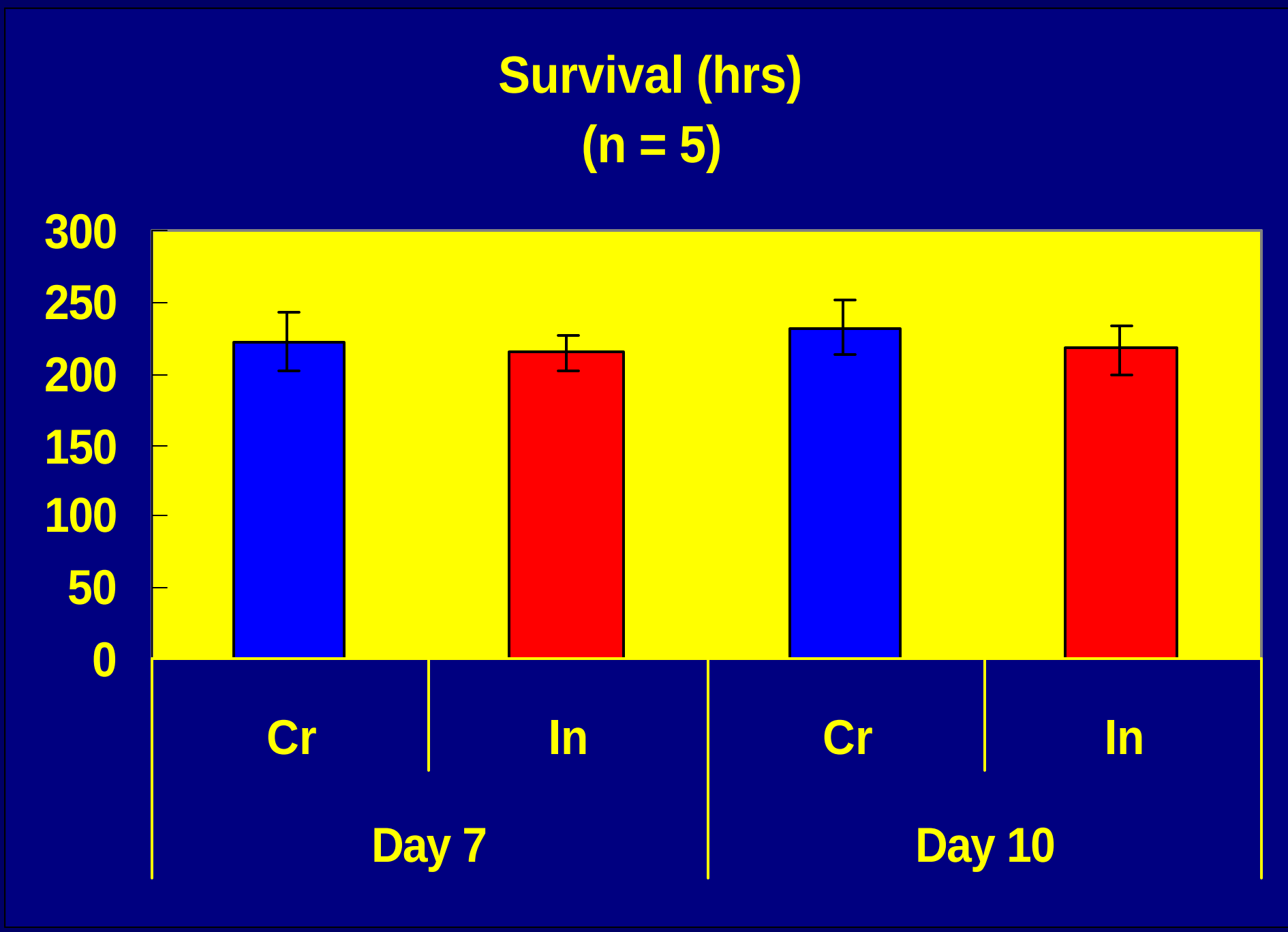
I (ES)	F	45.6	37.58	192.4	195.2	46.01	37.38	206.8	187
MEAN		50.83	53.59	223.28	214.23	51.21	54.39	231.73	217.62
Std Dev		14.64	17.45	19.14	10.68	14.10	18.01	17.84	15.43

Snyder Lab Recovery and Survival Data (n=5)

Subject	Sex	day 7 recovery (%)		day 7 survival (hrs)		day 10 recovery (%)		day 10 survival (hrs)	
		Cr	In	Cr	In	Cr	In	Cr	In
D (DF)	F	51.01	63.09	227.6	221.6	53.53	63.55	245.3	217.9
E (DP)	F	68.9	69.88	250.2	219.5	65.6	69.1	257.7	224.1
F (GC)	M	60.7	68.19	225.3	224.8	62.23	69.93	230.2	223.8
H (GM)	F	52.79	55.55	214.1	210.5	53.7	59.6	221.8	229.3
I (ES)	F	45.6	37.58	192.4	195.2	46.01	37.38	206.8	187
MEAN		55.8	58.858	221.92	214.32	56.214	59.912	232.36	216.42
Std Dev		9.11	13.14	21.07	11.94	7.78	13.28	19.87	16.93

Recovery (%)
(n = 5)




Survival (hrs)
(n = 5)
300
250
200
150
100
50
0
Cr
In
Cr
In
Day 7
Day 10

Summary

- Use of *en-tube* radiolabeling with 111-In or 51-Cr is feasible even for low Cr 51 LE and 2”xtal
- L.E. - ? independent of platelet ct / technique
- Volunteer donor with low normal platelet count may not prove problematic for 51-Cr labeling
- High wastage ($) of 51-Cr is a consideration
- Sampling for 10 days post injection is equivalent to sampling for 7 days post injection
- Additional data needed to determine % of control value (multi-center studies)

Snyder Lab

- Laurene Baril - Research Manager
- Tammy Corda - Research Associate
- Dottie Dincecco - Research Associate
- Eileen Smith - Nuclear Med Specialist